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Solute and particle retention in a small grazing antelope, the blackbuck (*Antilope cervicapra*)

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Digesta retention patterns have been suggested to play a major role in ruminant diversification. Two major digestion types have been proposed, termed 'cattle-type' and 'moose-type', that broadly correspond to the feeding categories of grazers and intermediate feeders on the one, and browsers on the other hand. We measured and calculated the mean retention time (MRT) of a solute and a particle (<2 mm) marker in the gastrointestinal tract (GIT) and the reticulorumen (RR) of a small grazer, the Indian blackbuck (*Antilope cervicapra*, n = 5, body mass of 26 ± 4 kg) and an intermediate feeder, the nilgai (*Boselaphus tragocamelus*, n = 5, body mass of 168 ± 21 kg). MRT_{solute} and $MRT_{particle}$ were 29 ± 4.1 h and 60 ± 6.6 h in blackbuck and 28 ± 2.5 h and 54 ± 8.9 h in the nilgai for the GIT, and 14 ± 1.7 h, 45 ± 5.0 h, 19 ± 2.0 h and 45 ± 8.4 h for the RR, respectively. With a selectivity factor (SF, the ratio of $MRT_{particle}$ to MRT_{solute}) in the RR of 3.2 ± 0.28 for blackbuck and 2.3 ± 0.36 for nilgai, both species are clearly in the category of 'cattle-type' ruminants. In particular, the high SF_{RR} of blackbuck, in spite of its small body size, is remarkable, and leads to specific predictions on the RR anatomy of this species (such as a particularly large omasum), which can be tested in further studies. The adaptive value of a high SF_{RR} is mainly considered as an increase in microbial productivity in the RR.

1. Introduction

Theory of ruminant feeding types, characterised by their preferred food plants and the correlated morphology of the gastrointestinal tract (GIT) (Hofmann, 1989), has been developed on East African ruminants. However, ruminants also evolved to considerable diversity in other geographic regions, such as India with a prominent fauna of cervids and bovids. Among the most interesting in terms of feeding ecology and morphophysiological adaptations of the GIT is the Indian blackbuck (*Antilope cervicapra*), the only representative of the Antilopinae considered a strict grazer. Grasses represent its major food resource under free-ranging conditions (Schaller, 1967; Chattopadhyay and Bhattacharya, 1986; Goyal et al., 1988; Henke et al., 1988; Jhala, 1997; Solanki and Naik, 1998), but also out of their natural habitat, blackbuck prefer grass if available (Henke et al., 1988; Frisina and Frisina, 1997). The blackbuck is small and with 25–35 kg represents the lower end of the body mass (BM) continuum of grazing ruminants. Body mass has been discussed as an alternative explanation for patterns of ruminant digestive physiology. The negative correlation of BM and selectivity has been suggested to be a main driver of morpho-physiological adaptations, and typical grazer attributes to be reserved to larger grazing taxa only (Gordon and Illius, 1994; Robbins et al., 1995). However, despite its low BM, the feeding type classification of Indian blackbuck as a grazer (Hofmann, 1991) is supported by a set of morphological characters such as a comparatively large rumen (Henke et al., 1988), small salivary glands (Hofmann et al., 2008), large masseter muscles (Clauss et al., 2008a), distinct ruminal papillation pattern (Clauss et al., 2009c), and prominent reticular crests (Clauss et al., 2010a). However, further evidence from digestive physiology is still missing. Various feeding studies have been performed with blackbuck (Pathak et al., 1992; Garg et al., 2002; Das et al., 2012), but patterns of digesta passage such as the ratio of the mean retention time of small particles and solute/fluid in the gut ($MRT_{particle}/MRT_{solute}$, the 'selectivity factor' SF) have not been quantified in this species to date. Such traits have been linked to ruminant feeding types (Hummel et al., 2005; Clauss et al., 2006), and more recently have been used to characterise two prototypes of reticulorumen (RR) physiology: the 'moose-type' with a comparatively low, and the 'cattle-type' with a relatively high ruminal fluid throughput (Clauss et al., 2010b). If the SF_{RR} (selectivity factor for the reticulorumen) is considered to reflect a digestive strategy of certain

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ruminants, it likely bears some physiological/nutritional advantage. A high SF_{RR} ('cattle-type') has been regarded as an indication for a distinct stratification of rumen contents, with a dry 'fibre mat' on top of a more fluid layer and a low-viscosity rumen fluid, as for example in addax (*Addax nasomaculatus*) (Hummel et al., 2008; Clauss et al., 2009b) or cattle (*Bos taurus*) (Hummel et al., 2009; Lechner et al., 2010). In contrast, species with a low SF_{RR} ('moose-type') have been reported to have comparatively homogenous rumen contents with a more viscous rumen fluid, such as roe deer (*Capreolus capreolus*) (Behrend et al., 2004; Clauss et al., 2009a) or moose (*Alces alces*) (Clauss et al., 2009b; Lechner et al., 2010). Furthermore, these digestion types differ in the presence ('cattle-type') or absence ('moose type') of a distinct gas dome in the rumen (Tschuor and Clauss, 2008). A higher microbial output has been shown for fermentation chambers with a high dilution rate (Owens and Goetsch, 1986), which would be increased by a high SF_{RR} in ruminants.

Based on the reported distinct difference in papillation of the dorsal and the atrium rumen mucosa, which is assumed to be directly linked to the difference between the passage of fluids and small particles (Clauss et al., 2009c, 2011b; Hebel et al., 2011), we expected a high SF_{RR} in blackbuck of about 2.0 or higher. Another Asian ruminant, the nilgai (*Boselaphus tragocamelus*; 150–200 kg BM), was also included in the study. As for the blackbuck, to date no data on retention times of solutes and particles exist for this ruminant. It is generally classified as intermediate feeder, seasonally including considerable amounts of browse such as Acacia and other trees in its diet (Schaller, 1967). Its food choice in its Asian habitat is described as including grass, but being clearly dominated by browse (Dinerstein, 1979; Khan, 1994; Sankar et al., 2004); free-ranging animals in Texas display higher proportions of grass but are still clearly identified as intermediate feeders (Sheffield, 1983), a classification that was also confirmed in experimental feeding plots in India (Solanki and Naik, 1998). In consequence, a lower SF_{RR} was expected for nilgai compared to blackbuck.

2. Methods

Five adult blackbuck antelopes (2 males, 3 females) and five adult nilgais (2 males, 3 females) at the Al Wabra Wildlife Preservation (AWWP), Qatar, were used in the study. They had been adapted to a diet of grass hay (Rhodes grass, *Chloris gayana*) and fresh alfalfa (*Medicago sativa*) for three weeks. While grass hay was provided ad libitum, fresh alfalfa was fed restricted to amounts of 0.16 kg (as fed) in blackbucks and 2.9 kg (as fed) in nilgai (dry matter content of fresh alfalfa was 15%; the proportion of grass hay in the ingested diet was 94% for blackbuck and 74% for nilgai on a dry matter basis). Blackbucks were kept individually for an adaptation period of 3 days in climatized indoor shifting pens that allowed feeding and faecal collection without direct contact to the animals, after which the one-week recording of individual food intake and faecal collection began in these pens. Nilgais were kept individually in outdoor enclosures that provided shade as well as shelter against wind and rain, and that were large enough to allow faecal collection without causing nervousness in the animals and having to shift animals. Animals had access to drinking water ad libitum. The food intake of the animals was quantified by weighing the amount of hay and fresh alfalfa offered and the amount of hay left over on a daily basis for six consecutive days (fresh alfalfa was always consumed completely). Animals were weighed at the end of the experiment (Table 1).

Nutrient contents of the forages were for Rhodes grass: ash 13.0% DM; crude protein (CP) 11.8% DM; neutral detergent fibre (ash corrected; NDFom) 68.3% DM; acid detergent fibre (ash corrected; ADFom) 34.0% DM; ether extracts (EE) 2.0% DM and for alfalfa: ash 15.1% DM; CP 22.4% DM; NDFom 38.9% DM; ADFom 26.7% DM; EE 2.7% DM. Blackbucks had an average diet composition of CP 12.4 ± 0.2% DM, NDFom 66.7 ± 0.9% DM and ADFom 33.6 ± 0.1% DM, while it was CP 14.7 ± 0.8% DM, NDFom 60.3 ± 2.2% DM and ADFom 32.0 ± 0.5% DM in nilgai. All nutrient analyses were done according to VDLUFA (2012) (ash: method 8.1; NDFom/ADFom: method 6.5.1/6.5.2; CP (Dumas): method 4.1.2; EE: method 5.1.1).

Table 1
Body mass, food intake and digesta retention parameters in blackbuck (*Antilope cervicapra*) and nilgai (*Boselaphus tragocamelus*). Significant effects in bold.

	Blackbuck	Nilgai	p*
n	5	5	
BM (kg)	26 ± 4	168 ± 21	–
Dry matter intake (g/kg $BM^{0.75}$/day)	40 ± 8.2	35 ± 8.5	0.421
MRT_{solute} GIT (h)	29 ± 4.1	28 ± 2.5	0.917
$MRT_{particle}$ GIT (h)	60 ± 6.6	54 ± 8.9	0.421
SF_{GIT}	2.1 ± 0.16	1.9 ± 0.25	0.310
MRT_{solute} RR (h)	14 ± 1.7	19 ± 2.0	**0.011**
$MRT_{particle}$ RR (h)	45 ± 5.0	45 ± 8.4	0.917
SF_{RR}	3.2 ± 0.28	2.3 ± 0.36	**0.016**

BM body mass, MRT mean retention time, GIT gastrointestinal tract, RR reticulorumen, SF selectivity factor (the ratio of $MRT_{particle}$ to MRT_{solute}).
* Test for differences between the species.

Dissolved cobalt(Co)-EDTA and chromium(Cr)-mordanted fibre (<2 mm) prepared from grass hay according to Udén et al. (1980) were used as markers for the fluid and the particle phase, respectively. A pulse-dose of the markers was fed to each animal and mixed into a handful of wheat bran. The latter was added to increase palatability and to guarantee the ingestion of the markers in a short time period. The marker was fed late in the afternoon and was well accepted. Blackbucks received approximately 0.4 g of Co-EDTA and 5 g of Cr-mordanted fibre and nilgais of 4 g and 30 g, respectively. Prior to marker feeding, three faecal samples were taken to analyse Co and Cr background levels. After marker feeding, faecal samples were taken regularly for 7 days, with intensive faecal sampling during the first two days and increasing time intervals subsequently; in the case of nilgai, sampling only occurred during daylight hours, but blackbucks were sampled also during the night (cf. sampling times indicated on the x-axes in Fig. 1, with evident night intervals missing for nilgai). Note that the equation used to determine mean retention times in this study is not affected by sampling interval (Van Weyenberg et al., 2006). A representative subsample of all defecations was stored frozen until drying at 60 °C and milling with a centrifuge mill (Retsch 2M1, 1 mm sieve; Retsch, Haan, Germany).

Marker analysis followed the procedure outlined by Behrend et al. (2004) and Hummel et al. (2005); a wet ashing with sulfuric acid (72%) was followed by atomic absorption spectroscopy. From the resulting faecal marker concentrations, mean retention time in the GIT was calculated according to Thielemanns et al. (1978)

$$MRT = \sum(t_i * dt * c_i) / \sum(dt * c_i)$$

with t_i = time after marker application (h), dt = time interval represented by marker concentration (calculated as $(((t_{i+1} - t_i) + (t_i - t_{i-1})) / 2)$, and c_i = faecal marker concentration at time i (mg/kg DM)). The middle of the sampling intervals was used as t_i.

MRT in the RR was estimated following Lechner-Doll et al. (1990): MRT_{solute}RR is determined by estimating the rate constant of the descending part of the marker excretion curve via an exponential equation:

$$y = A * e^{-k * t}$$

with y = faecal marker concentration at time t (mg/kg DM), A = a constant, k = rate-constant (h^{-1}) and t = time after marker dosing (h); the reciprocal of k represents the MRT for the RR. $MRT_{particle}$RR is calculated based on the assumption that fluid and particles do not